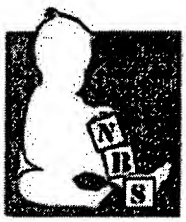

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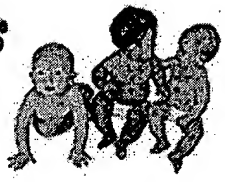

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A layperson's guide to **Tandem Mass Spectrometry and Newborn Screening**

Written By Dr. Donald H. Chace, Ph.D., M.S.F.S.

Recent advances in technology and clinical intervention have enabled clinical laboratories providing newborn screening services to improve testing and to expand testing to include additional treatable disorders. One of the major technical advances in newborn screening is the use of an analytical instrument known as a tandem mass spectrometer. Research and development in the newborn screening applications of tandem mass spectrometry were started in the early 1990's and they continue today. More than 20 disorders of body chemistry can be detected in a single analysis of a small blood sample that is collected on a special paper during the first few days of life. The tandem mass spectrometer and how it is used in a newborn screening laboratory is described below. It is hoped that information provided here will create a better understanding of this new powerful technology and the ways that this technology can be used to prevent disabilities and premature death.

• What is a mass spectrometer?

A tandem mass spectrometer is one of several types of analytical instruments known as mass spectrometers. Mass spectrometers are used in many laboratories throughout the world to analyze thousands of compounds such as those present in our bodies, our environment, our medicines, manufactured materials, foods, poisons, and criminal evidence. Mass spectrometers can be thought of as instruments that weigh molecules. Of course, molecules are extremely small and cannot be weighed in the traditional sense on a scale. To give you an estimate of the size of a molecule of water, it would take approximately 60,000,000,000,000,000,000,000 water molecules to fill a tablespoon.

Chemists use a mass spectrometer to "electronically" weigh molecules. We refer to the weight of a molecule as its mass. Every molecule has a unique mass. The mass of water is 18, common table salt (sodium chloride) is 58, and sucrose (from sugar cane) is 342. In addition to identifying a compound by its mass, we can determine how much of the compound is present in the material that we are analyzing. The best analogy that can be used is *pocket change*. If you grab a handful of coins from your pocket or purse it may consist of pennies, nickels, dimes and quarters. You could sort these coins based on their unique weight and group them so you have perhaps dimes (the lightest coins), pennies, nickels and quarters (the heaviest coin). A mass spectrometer sorts molecules in a fashion analogous to sorting money. Notice that you could also determine how many coins of each type you have in your pocket after sorting into separate groups by counting each group. That is analogous to how a mass spectrometer can measure how much of a compound is present in a mixture.

• What is a "tandem" mass spectrometer?

A tandem mass spectrometer can be thought of as two mass spectrometers in series connected by a chamber that can break a molecule into pieces perhaps like a puzzle. This chamber is known as a collision cell. A sample is "sorted" and "weighed" in the first mass spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer.

Why do we need to use a tandem mass spectrometer? The blood samples that we screen hundreds of compounds in them, but we are interested in only a few dozen. It is not practical to identify every compound in the samples that we receive, so we look for compounds of interest to us that have diagnostic significance. Fortunately, the compounds in the blood samples that we analyze have certain common and unique characteristics. These compounds are members of a chemical class or family such as amino acids or fatty acids.

• Types of tandem mass spectrometers

A tandem mass spectrometer is often abbreviated as Tandem MS or MS/MS. Sometimes you will also notice the abbreviation ESI (electrospray), FAB (fast atom bombardment) and LSI (liquid secondary ion) before the tandem MS. These terms indicate the way the compound is placed into the tandem mass spectrometer. Photos of two kinds of tandem mass spectrometers using in a newborn screening laboratory are shown below.

• How is a tandem mass spectrometer used in a newborn screening laboratory?

The compounds in the blood of infants that we test using tandem mass spectrometry are known as amino acids and acylcarnitines. Amino acids are the building blocks of proteins that become the important parts of our tissues, muscles, organs, blood. Carnitine is a transportation system for fats in and out of the cell's energy factory, the mitochondria. When a fat (as a fatty acid) is attached to carnitine it is called an acylcarnitine. We often identify acylcarnitines by the size of the fat molecule attached to it. These may be categorized simply as short, medium and long chain fats or denoted by a combination of letters and numbers. For example, the important medium sized fat attached to carnitine, that is measured in the disorder MCAD (medium chain acylCoA dehydrogenase) deficiency, is an eight carbon fatty acid knows as octanoylcarnitine and is abbreviated C8. The tandem mass spectrometer can weigh this molecule and all of the other acylcarnitines as well and tell us how much is present. The results produced by the mass spectrometer display the data as vertical lines distributed across a horizontal axis (called a mass spectrum). Where the vertical line occurs in the spectrum identifies a compound's mass while the height of the line represents how much of the compound there is.

• Why are the measurements of amino acids and acylcarnitines important?

In inherited metabolic diseases, specific enzymes (catalysts) that helps facilitate the breakdown of amino acids or the conversion of fat to energy do not function. If a particular enzyme is not functioning, the breakdown of a compound by this enzyme (metabolism) to waste products does

not occur. In other instances, products are not produced that are important in generating fuel for the cell. Because the compound can not be metabolized, it will accumulate in the blood and tissues. The compound in excess then becomes a poison rather than a "normal substance." The tandem mass spectrometer, by measuring the amounts of amino acids or acylcarnitines in blood, can tell us using special computer software and expert medical interpretation, whether there is too much of the compound that we analyzed in the blood. This information is communicated to the pediatrician and together with other information will indicate what additional tests are needed to confirm the presence of an inherited disease. If confirmed, treatment of the inherited disorder is started. Treatment of these diseases most often involves changes in the diet that may include fat or protein restriction and/or special vitamin supplements. The pediatrician will probably collect blood samples occasionally to insure that a normal body chemistry is restored and if not, whether the diet must be changed to increase or decrease protein or fat intake.

Why use mass spectrometry?

Research has shown that tandem mass spectrometry can improve laboratory analyses because it is very specific in its identification of the compounds we are interested in, it is very accurate and can measure very small amounts of material with excellent precision. More importantly, it has the ability to measure more than one compound simultaneously in a single, two minute analyses. Other newborn screening tests do not currently exist for measuring acylcarnitines and so tandem mass spectrometry must be used to detect metabolites produced in MCAD deficiency.

For amino acid disorders such as PKU, tandem mass spectrometry has been shown to reduce the false positive rate (false alarms) for this disorder by more than 10 fold compared to the best alternative method available. In addition, much rarer disorders such as MSUD (maple syrup urine disease), citrullinemia, VLCAD (Very Long Chain Acyl CoA dehyrdogenase) deficiency, GA-I and GA-II, propionic academia and methylmalonic acidemia can be detected, simultaneously.

• What are the limitations of tandem mass spectrometry?

Tandem mass spectrometers are complex instruments. The methods used to prepare the samples for analysis by mass spectrometry require specialize reagents. Although tandem mass spectrometry is often called a simple blood test, it is not a simple method. Tandem mass spectrometry requires several expert scientists to perform the analyses and medical experts to interpret the large amount of clinical data produced from the analysis of a blood sample. The instruments are expensive and in order to achieve appropriate cost/benefit for analyses of blood samples, it is necessary to screen many tens of thousands of samples per year. In addition, tandem mass spectrometry can not affect problems with blood sample collection and delivery to a laboratory nor the speed at which results are communicated to the physician. Mass spectrometry is a tool that could expand the disorders screened for in qualified newborn screening laboratories having sufficient experience with the technology.

• The Future

In excellent laboratories, tandem mass spectrometry will become a powerful tool to expand newborn screening and to significantly improve newborn testing for many diseases. Tandem mass spectrometry and other forms of mass spectrometry will likely be used to analyze other treatable disorders or to improve existing tests so that more accurate and more comprehensive testing will be available. We are at a new horizon in screening and with new treatments available and under development, our future generations will benefit.

This article has been submitted by:
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Dr. Chace is one of the primary investigators that developed the newborn screening application of tandem mass spectrometry. He was an assistant medical research professor in the department of Pediatrics at at Duke University Medical Center from 1990-1997. He joined Neo Gen Screening (now Pediatrix Screening) in May of 1997 to develop high throughput mass spectrometry for screening hundreds of samples a day as well as to develop computer interpretation systems to assist physicians in diagnosing metabolic disorders such as MCAD deficiency. During the Year 2000, over 1 million newborn samples will have been analyzed at Neo Gen Screening (now Pediatrix Screening). He is currently working with the CDC to develop quality assurance systems for newborn screening of inherited metabolic diseases as well as new mass spectrometry methods to detect additional diseases to improve the quality of life for newborns and their families.

For additional information on Tandem Mass Spectrometry click on Expanded Newborn Screening Using MS/MS

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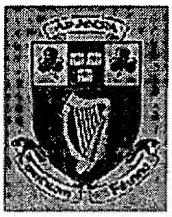

Up

Tandem MS or MS/MS is used for structure determination of molecular ions or fragments. In Tandem MS, the ion of interest is selected with the first analyzer (MS-1), collided with inert gas atoms in a collision cell, and the fragments generated by the collision are separated by a second analyzer (MS-2). In Ion Trap Mass Spectrometers such as our LCQ the experiments are carried out in one analyzer, and the various events are separated in time, not in space. The information can be used to sequence peptides and small DNA/RNA oligomers, to determine structure and connectivity of polysaccharides, to determine the position and structure of fatty acids in complex lipids, and to carry out other structure determinations. Useful structural information can be gained from simgle compounds ionised by ESI which typically contain only one major peak (M+H)+

MS-MS is available on the LCQ

Home Up

Send mail to peter.caplan@ucd.ie with questions or comments about this web site.
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The University of Bristol, School of Chemistry

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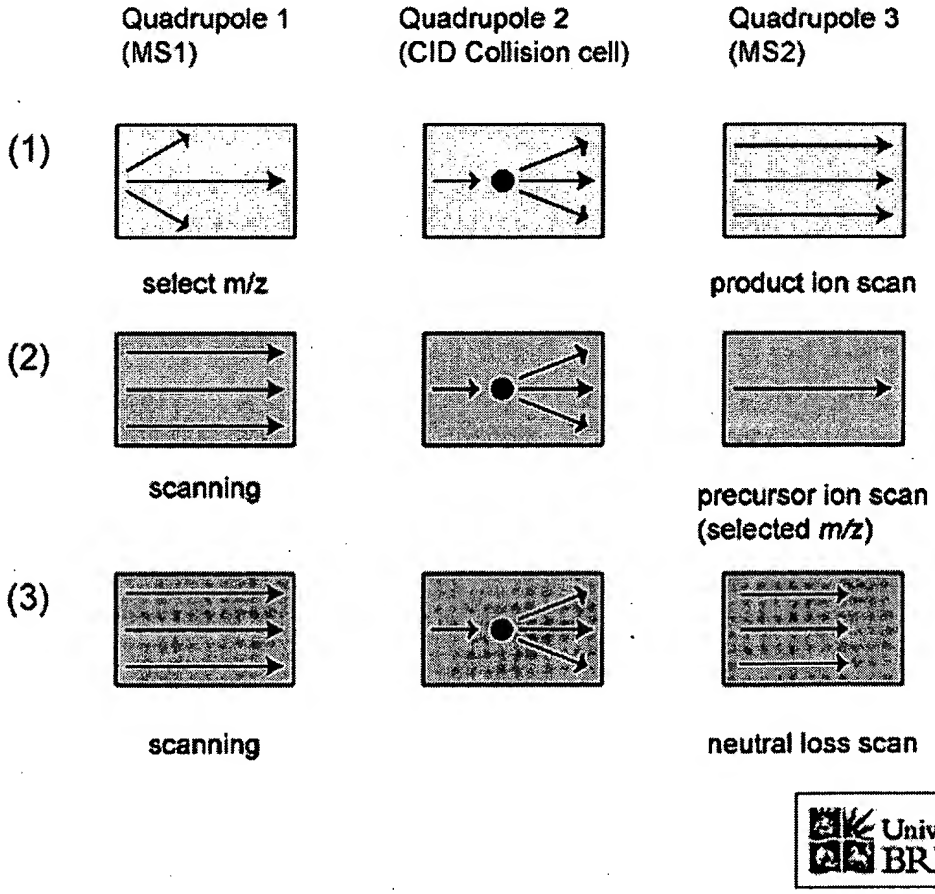
MASS SPECTROMETRY RESOURCE

Tandem Mass Spectrometry (MS/MS)

In the "classical" ionisation methods for mass spectrometry, like EI and CI, spectra usually contain a good amount of fragment ions that can be used to help confirm or elucidate chemical structures. In the more modern methods of ionisation, like ESI or MALDI, spectra often only contain the ionised molecule with very little fragmentation data and consequently the spectra are of little use for structural characterisation. In these cases, induced fragmentation is required using collision induced dissociation (CID) and tandem mass spectrometry (MS/MS). One of the most commonly available tandem mass spectrometers is the triple quadrupole (QQQ) instrument. There are many other varieties and configurations of tandem instrument and although this page will describe how a triple quadrupole works - most of what is said will apply generally with little modification.



In a triple quadrupole mass spectrometer, there are several types of experiment that can be performed. The figure shows a schematic representation of three common types of MS/MS experiment.

(1) Product ion scan. In this case, the precursor ion is focussed in Q1 and transferred into Q2 - the collision cell

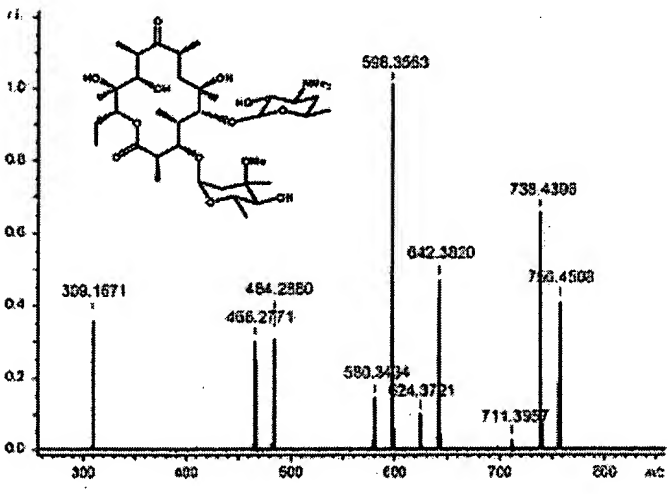
- where it interacts with a collision gas and fragments. The fragments are then measured by scanning Q3. This results in the typical MS/MS spectrum and is the method most commonly employed with ESI ionisation and/or LC-MS.

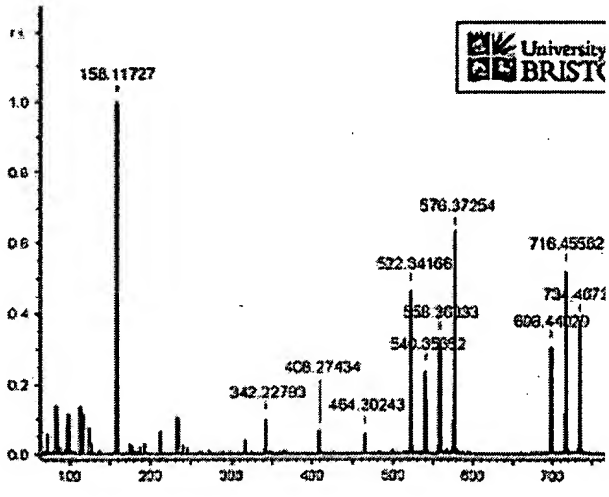
(2) Precursor ion scan. In this case Q3 is held to measure the occurrence of a particular fragment ion and Q1 is scanned. This results is a spectrum of precursor ions that result in that particular product ion - this is especially useful when used with EI or CI ionisation and/or GC-MS.

(3) Neutral loss scan. In

this case Q1 is scanned as in (2) but this time Q3 is also scanned to produce a spectrum of precursor ions that undergo a particular neutral loss. Again this mode is especially useful for EI and CI ionisation.

In ESI-MS/MS it is often desirable to fragment more than one precursor ion of the same compound. In the example below, the two spectra show the ESI-MS/MS of the sodiated $[M+Na]^+$ and the protonated $[M+H]^+$ precursors respectively of the antibiotic erythromycin A. These two spectra show a very different series of fragment ions that are the result of different gas-phase chemical processes. The Na cation is able to stabilise some structures through chelation, which enables some of the fragment routes, whereas the proton is able to initiate chemistry especially with heteroatoms (like oxygen or nitrogen). In this example it is not possible to say where the cations are in the molecule, and in fact it is quite likely that each route is initiated by the cation being in a different location. This extra data obtained in this way is essential in structural elucidation studies. More information is contained in the research section of this web site.





Two-Dimensional Techniques - Gas Liquid Chromatography & Mass Spectrometry

A truly multidimensional system is one where a separation dimension must be coupled with a spectroscopic dimension and the following conditions are thus applicable: [1] The components of a mixture are subject to two or more separation steps, in which their displacements depend on different factors and [2] when two or more components are separated in any single step, they always stay separated until the total separative operation is finished.

High Performance Liquid Chromatography-Gas Chromatography (HPLC-GC)

Multidimensional liquid chromatography-gas chromatography (LC-GC) combines the selectivity of LC with the high efficiency and sensitivity of GC, thus giving a relatively high peak capacity. The advantages of on-line LC-GC are: [1] less sample required, [2] no sample workup, [3] fully automated sample pre- or post-treatments are possible as well as [4] no evaporation or dilution is necessary. Coupling of LC-GC is not a trivial issue, as both the LC and GC operate in phases that are in two different physical states. Normal-phase liquid chromatography (NPLC) is more easily coupled with GC than reversed-phase liquid chromatography (RPLC), and thus the eluent is usually a non-polar volatile solvent. In 1989 the first fully automated LC-GC instrument was pioneered for commercial use. Since then, LC-GC has been shown to be applicable to many areas of analysis, including industrial samples and petrochemicals, environmental samples and biological and pharmaceutical samples.(1,2)

Comprehensive Two-Dimensional Gas Chromatography (GCxGC)

Comprehensive Two-dimensional gas chromatography (GCxGC) has been shown through different types of research to be applicable to many areas of analysis. This type of two-dimensional gas chromatography was first described 12 years ago, and since then the number of publications in the field have grown rapidly. GCxGC is now considered the main technique used to provide a very high separation power, as well as providing an enhancement of sensitivity and structured chromatograms. It is a multidimensional technique in which the resolving power of two or more different columns is applied to some or all of the components in a sample. Many advantages are associated with this technique including: [1] complex samples can be separated

into many distinct peaks (providing an alternative for group-type analyses), [2] superior resolution when compared to conventional GC, [3] boiling-point distributions for different classes of compounds concurrently, and [4] acts as a means for rigorous validation of existing and recently developed techniques.(3)

Gas Chromatography-Mass Spectrometry (GC/MS)

Gas Chromatography-Mass Spectrometry permits the analysis of two or more compounds eluted simultaneously. This can be achieved in two ways: either direct coupling or via an open-split coupling.

Liquid Chromatography-Mass Spectrometry (LC/MS)

Liquid Chromatography-Mass Spectrometry is far more difficult to achieve than GC/MS. Limitations include the the limited detection procedures. It does though have the advantage that it is connected with isolation, pre-purification and analysis in a single chromatographic step. Therefore the most applicable LC/MS interface for a specific application must be selected, based on analyte polarity.

Tandem Mass Spectrometry (MS/MS)

Tandem Mass Spectrometry is where two mass analyzers are used: one for selecting the precursor ion from the ions created in the ion source and the other for analyzing the product ions after the collisions. It is fairly useful in quantitative environmental and bioanalysis

References:
(1) Grob, K (1991). "On-line coupled LC-GC." Huthing, Heidelberg, Germany.
(2) Mondello, L., G. Dugo, et al. (2003). "Recent applications in multidimensional chromatography." 1: 2.
(3)Beens, J., J.Blomberg (2000). "Comprehending Comprehensive Two-Dimensional Gas Chromatography". 23: 2.

Reference Sites - of at least 1

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